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Reactive Site Binding

FIG. 1A

Chemical reaction to be catalyzed

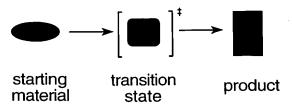


FIG. 1B PROfusion™ affinity binding to transition state analog

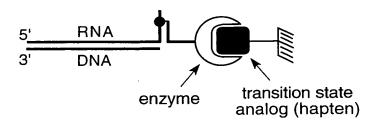
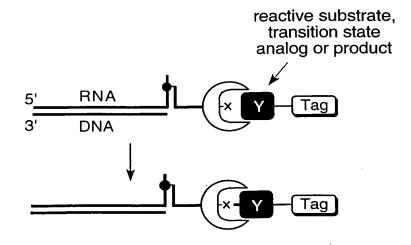


FIG. 1C

Covalent binding to reactive substrate, transition state analog, or product



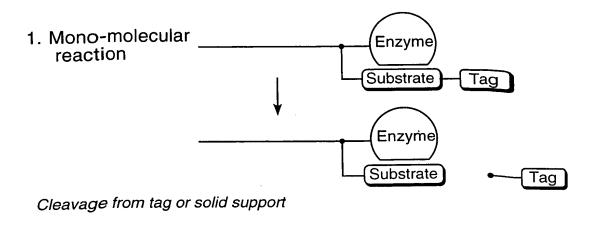
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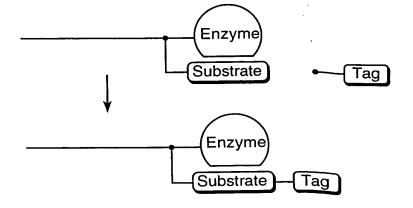
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Enzyme-Substrate Chimeras

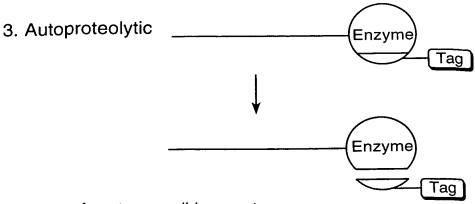
FIG. 2



2. Bi-molecular reaction



Attachment to solid phase, reaction with biotinylated substrate followed by capture on streptavidin resin, product immunoprecipitation with suitable antibody or gel-electrophoretic separation of modified and unmodified fusion (or cDNA portion)



Release from tag or solid support

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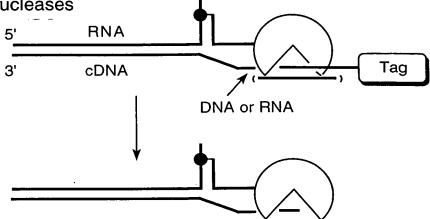
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Nucleases

FIG. 3

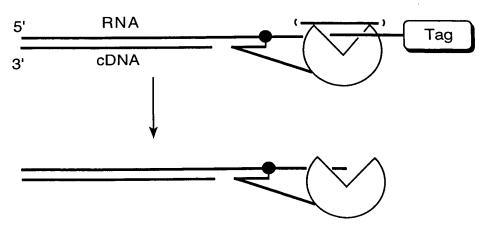
- DesoxyribonucleaseRibonuclease
- Restriction endonucleases



RNA - protein fusion

PROfusion™ DNases or endonucleases promote their self-cleavage from a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence. Similarily, this method can be used to alter the restriction site specificity of restriction enzymes after mutagenesis.

Ribonuclease



RNA-protein fusion

PROfusion™ DNases promote their self-cleavage from a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence.

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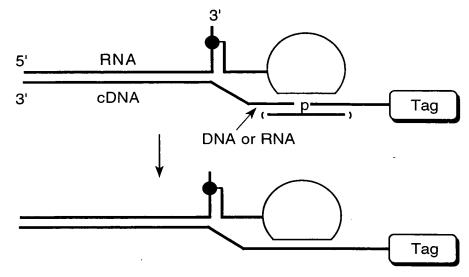
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Ligases

FIG. 4

- DNA ligase
- RNA ligase

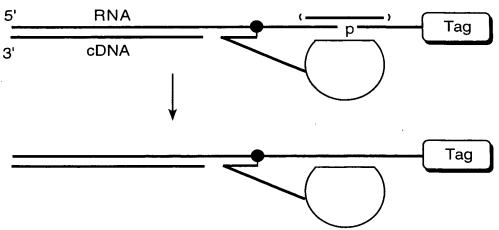


RNA-proteinfusion

PROfusion[™] DNases or RNA ligases catalyze their attachment to a tag or solid support. The use of the second strand is optional. Sequence-specific cleavage can be achieved through the choice of the target sequence. The second substrate is either directly attached to the solid phase, or e.g. biotinylated to allow capture with immobilized streptavidin. Alternatively, the size-difference between precursor and product may be used for electrophoretical separation.

RNA ligase

ः रह्ममुद्धाः राष्ट्राणानसम् द्वमुन्ना सम्प्राम्यम्यास्यस्य



RNA-proteinfusion

PROfusion™ RNA ligases catalyze their attachment to a tag. Similar considerations as for DNA ligases apply.

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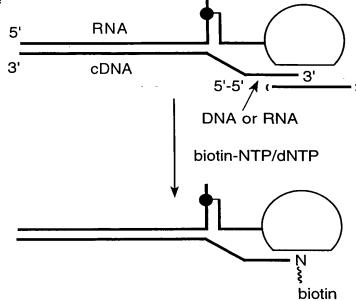
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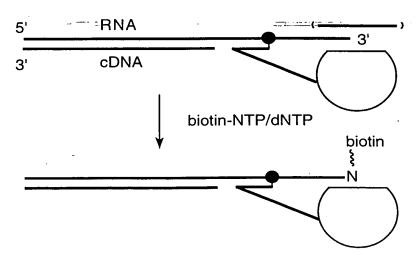
Polymerases and Terminal Transferases

FIG. 5

- Terminal transferase
- DNA polymerase
- RNA polymerase
- Reverse transcriptase



- Terminal transferase
- RNA polymerase
- Reverse transcriptase



RNA-proteinfusion

PROfusion capture through attachment of biotinylated nucleotide triphosphates. For the selection of polymerase enzymes a second strand must be used. Following reaction, the modified PROfusion can be captured with streptavidin resins.

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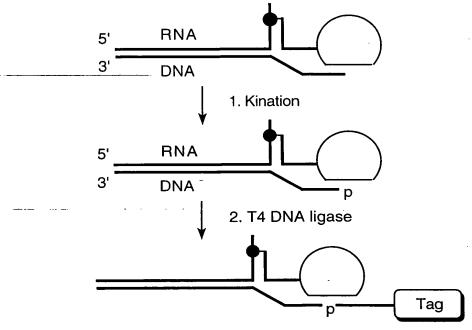
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Kinases and tRNA Synthetases

FIG. 6

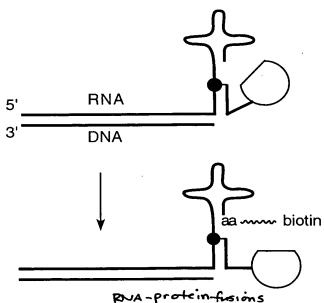
• Polynucleotide Kinase



RUA-protein-fusions

After phoshorylation, the kinase PROfusionsTM become substrates for ligation to allow the physical separation from the unmodified precursor.

• tRNA synthetase



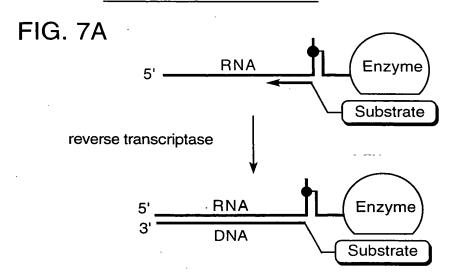
Attachment of biotinylated amino acids through PROfusions^{IM} with tRNA synthase activity. Successfully modified molecules may be captured on streptavidin supports. Note that the tRNA domain may also be attached to the cDNA portion.

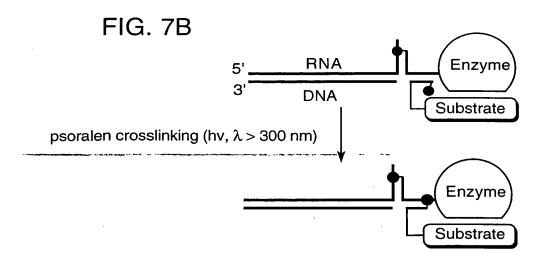
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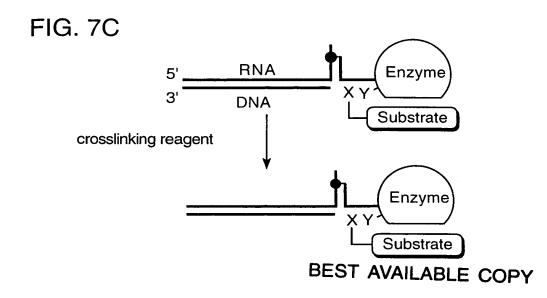
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Substrate Attachment







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3. Translation & Fusion Formation 2. Transcription 1. RT / PCR mRNA mRNA mRNA mRNA Induction of Cleavage C-Extein C-Extein C-Extein C-Extein Immobilization Elution Intein Intein Intein Intein N-Extein N-Extein N-Extein BEST AVAILABLE COPY

Applicant(s): KURZ et al.
Filing Date: December 1, 2003 Serial No.: Page 9 of 9 Customer No.: 31020 9/9 3. Translation & Fusion Formation 2. Transcription 1. RT / PCR N-terminal extein capture (removal) of unligated product, optional) mRNA mRNA mRNA mRNA Immobilization C-Extein C-Extein C-Extein C-Extein Induction Elution N-Extein N-Extein Intein N-Extein Intein BEST AVAILABLE COPY

mRNA

C-Extein

Intein

N-Extein

FIG. 9

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